Peer Review
Experimental Toxicology Division
N H E E R L

WATER

Pharmacokinetic Studies of Candidate Contaminant List Dichloropropanes and Dichloropropenes Using In Vitro and In Vivo Gas Uptake Systems

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INTRODUCTION

- In 1996, amendments to the Safe Drinking Water Act (SDWA) required that the U.S. Environmental Protection Agency (EPA) create a list of unregulated water contaminants that could eventually require regulation under the SDWA. In response to these amendments, the Contaminant Candidate List (CCL) was created.
- In 1998, the haloalkanes 1,3-dichloropropane, 2,2-dichloropropane, and 1,1-dichloropropene were placed on the CCL and designated high priority for research, in large part based on structural analogy to the well-studied rodent carcinogen 1,2-dichloroethane.

OBJECTIVE

- Estimate metabolic rate parameters for CCL, dichloropropanes and dichloropropenes and compare their metabolism to structurally similar carcinogens.
- Evaluate the consistency of results obtained using in vitro and in vivo methodologies to estimate metabolic rate parameters.

METHODS

- An automated equilibrium headspace technique utilizing gas chromatography was developed to determine rates of metabolism of volatile chemicals using liver cytosol and microsomes.
- Additional dihaloalkanes (1,2-dichloroethane, 1,2-dichloropropane, 1,4-dichlorobutane, 1,2-dibromoethane, 1,2-dibromopropane, 1,4-dibromobutane) were evaluated to assess structure-activity relationships.
- Parallel experiments were performed using the traditional in vivo gas uptake system to estimate metabolic rate parameters for 2,2-dichloropropane and 1,1-dichloropropene and to obtain data to further evaluate the comparative performance of the in vivo and in vitro systems.

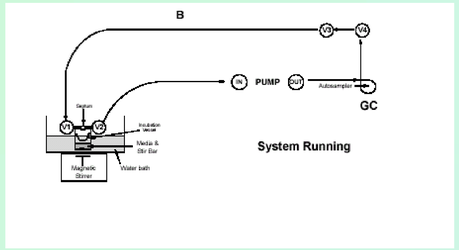
RESULTS

- In general, brominated dihaloalkanes were eliminated from rat cytosol faster than chlorinated dihaloalkanes, reflecting the expected halide order of reactivity with GSH (Br>Cl).
- Rates of GSH conjugation were proportional to a,w-haloalkane chain length.
- The in vitro system is an efficient and flexible tool for screening and prioritizing chemicals based on metabolic reactivity.

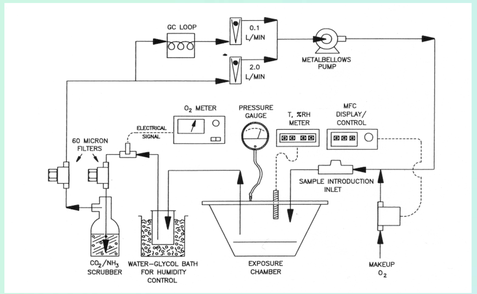
Table 1. Kinetic parameters of P450-dependent metabolism of haloalkanes catalyzed by rat liver microsomes.

Compound	Conc. Range (ppm)	K_1 (h^{-1})	P (liquid:air)	V_{max} (nmol/h/mg)	K_m (μM)
1,3-DCP	0.5-220	4.0	8.3	30.3	1.09
1,4-DCB	5-75	4.9	8.5	33.9	1.67
1,2-DBE	1.5-75	5.0	8.8	26.2	2.47
1,3-DBP	4-210	5.8	10.3	37.3	5.25
1,2-DCP	2.5-80	3.4	5.9	42.9	5.11
1,2-DCE	2.5-80	3.3	5.4	48.5	14.1
2,2-DCP	3-30	0.5	0.8	n.d.	n.d.
1,1-DCPe	3-98	2.6	1.6	56.8	2.51

In Vitro System Schematic Diagram

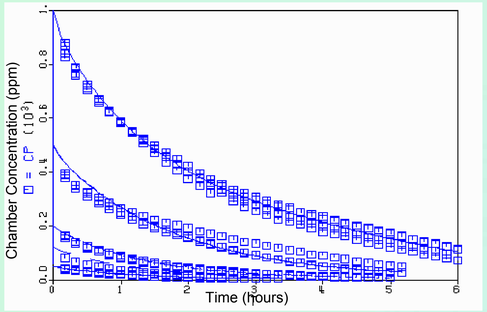


In Vivo System Schematic Diagram



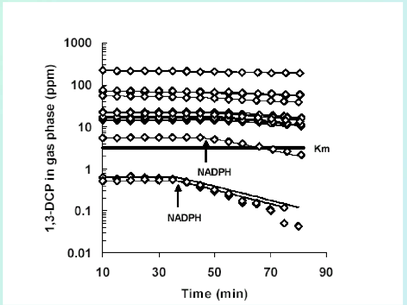
Principles of operation of the two systems are similar: A single bolus of chemical is injected into the system at the start of exposure and decline in chemical concentration is measured by gas chromatography. Loss in the chamber or headspace occurring after equilibration is related to metabolism. The chamber must be leak free in order to measure changes in chemical concentration accurately. Metabolic parameters can be estimated from the experimental data using a kinetic or physiologically based pharmacokinetic model.

In Vivo Gas Uptake – 1,1-Dichloropropene



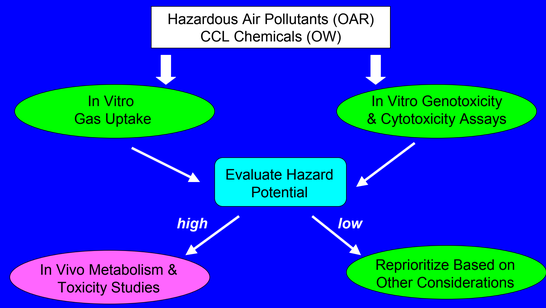
Disappearance of 1,1-dichloropropene from a closed chamber holding single male CD rat (n=4/initial concentration). The data (□) were simulated (smooth curves) by optimizing the metabolic constants (VmaxC, K_M) in the PBPK model. The optimized VmaxC and K_M are 5.7 mg/hr/kg and 0.3 mg/L, respectively.

In Vitro Gas Uptake – 1,3-Dichloropropane



Concentration-time courses of 1,3-DCP in the gas phase of the in vitro closed system with incubation medium containing rat liver microsomes (0.72 mg protein /ml, pH 7.4, 37°C). The apparent V_{max}= 30.3 nmol/h/mg microsomal protein. The apparent K_m =1.09 mM in the incubation medium and the corresponding K_m in the gas phase = 3.2 ppm. 1,3-DCP was not volatile enough to run in the in vivo gas uptake system.

Role of the In Vitro Gas Uptake System in Predictive Toxicokinetcs & Hazard Identification



CONCLUSIONS & IMPACT

- The metabolic rate constants determined for these halolalkanes are critical parameters for PBPK models used in risk assessments for the program offices.
- In Vitro System Advantages
  - ➢Fast throughput
  - ➢Ease of operation
  - ➢Evaluate cytosolic and microsomal metabolism
  - ➢Use of human microsomes and cytosol
  - ➢Greater range of chemical volatility and reactivity
- In Vivo System Advantages
  - ➢Respiratory physiology endpoints (plethysmography)
  - ➢Toxicology endpoints (telemetry, enzymology)
- The two technologies are complimentary and maintained as a core resource for PKB and other divisions within ORD/NHEERL.
- The in vitro gas uptake system has a pivotal role in predictive toxicokinetics and screening that will result in more efficient experimental design and reduced animal use.

FUTURE DIRECTIONS

- Evaluate and refine in vitro to in vivo extrapolation methods
- Predictive toxicokinetcs
- Database of metabolic rate parameters for HAP and CCL chemicals in rats and humans

SOLVING AGENCY PROBLEMS